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The Identification of Phytocompounds from *Limonia acidissima* L. Fruit:A Short Report and Updated Review-Focusing on Antihyperuricemic and Antiarthritic Activities

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ABSTRACT

The side effects of using anti-gout and anti-inflammatory drugs in patients with hyperuricemia and gouty arthritis are the reason to get alternative, safer herbal medicines. To answer the call from the Indonesian government to explore the natural product potential as treatment modalities against various diseases, we performed an investigation on *Limonia acidissima* L. fruit**.** Extraction of the *Limonia acidissima* L. fruit was carried out by means of maceration using different solvents (n-hexane, ethyl acetate, ethanol, and methanol), performed sequentially. Phytoconstituents of each extract were further determined by using Gas Chromatography – Mass Spectroscopy (GC-MS). The results revealed that *L. acidissima* fruit is rich with fatty acids including oleic acid, n-hexadecanoic acid, palmitoleic acid, and octadecanoic acid. Several important plant sterols, such as sitosterol, stigmasterol, ergost-5-en-3-ol, (3beta.), were also found in the extract. Updated literature review confirmed the potential of *Limonia acidissima* L. fruit application as phytomedicine. To conclude, we recommend further investigation on the *L. acidissima* fruit potentials as antihyperuricemic and antiarthritic therapies.

Keywords: *Antioxidant, phytoconstituents, inflammation, Limonia acidissima,uric acid*

1. INTRODUCTION

Hyperuricemia is joint inflammation due to the accumulation and deposition of monosodium urate which can cause various signs and symptoms such as pain, redness, swelling and joint stiffness [1]. Hyperuricemia is a major risk factorfor gouty arthritis [2] .The use of natural ingredients as alternative medicine for metabolic diseases has been massively campaigned by the government of the Republic of Indonesia through the slogan "back to nature". The agenda of this program include obtaining new bioactive compounds as well as to reveal the role of nutrients contained in plants in overcoming publichealth problems. Our research group has an experience in responding this call [3]–[5]. *Limonia acidissima* Linn (*L. acidissima*) is a plant originating from India and Sri Lanka and then spread to Indonesia. So far, it has been used to treat diarrhea and dysentery [6], diabetes [7], wound healing and antioxidants [8], hepatoprotective [9] and as a cosmetic ingredient [10]. Preliminary results of our research show that NIRS (Near-infrared spectroscopy) results of *L. acidissima* fruit from five districts in Aceh, Indonesia identified the presence of CH, OH, CHO and NH molecularbonds as the main components forming organic matter. This is related to the carbohydrate, acid, protein or fiber content of this fruit [11]. In this present work, we extracted *L. acidissima* fruit using sequential maceration using n-hexane, ethyl acetate, ethanol, and methanol solvents (non-polar to polar). Further, the extracts were analyzed using Gas Chromatography – Mass Spectroscopy (GC-MS) to reveal their phytoconstituents. To accompany the analysis results, we presented the updated literature review. This report is a part of project that aims to discover the antihyperuricemic and antiarthriticpotentials of *Limonia acidissima* Linn fruit.

2. METHOD

Plant specimen was taken from Aceh Besar, Aceh Province, Indonesia (5°29′11.3″N 95°25′40.3″E). The taxonomic

determination was carried out at the Herbarium Laboratory, Department of Biology, Universitas Syiah Kuala, Indonesia. Gass Chromatog-raphy-Mass Spectrometry (GC-MS) analysis of extracts of n-hexane, ethyl acetate, eth- anol, and methanol of L. acidissima pulp was performed on CG-MS-QP2020 NX with SH-Rxi-5Sil MC Cap.column with a size of 30 m x 0.25mm x 0.25 μm.

Prior to extraction, fruits of L. acidissima (12 kg) were separated from its peels and seeds, and subsequently oven- dried at 40°C. The sample was crushed into find powder. The maceration was performed on simplicial powder (2 kg) in a sealed container using n-hexane and sequentially partitioned using ethyl acetate, ethanol, and methanol. The maceration was repeated 3x24 h, while stirred occasionally. In each repetition, the fil-trate was separated using filter paper and concentrated with rotary evaporator (40°C; 30 rpm). This process yielded extract samples as much as 4.3 g (0.215%), 75 g (3.75%), 110.8 g (5.52%), and 55.6 g (2.78%) for n-hexane, ethyl acetate, ethanol, and methanol, respectively. Each extract sample was stored in a sealed container at 4°C until further use.

3. RESULTS AND DISCUSSION

3.1. Phytocompounds Identified

The phytochemical screening of *L. acidissima* fruit revealed the presence of flavonoids, polyphenols, alkaloids,tannins, saponins, and the absence of alkaloids mayer (Table 1).

Table 1. Qualitative phytochemical screening of *L. acidissima* extract

Phytoconstituents	Solvent	n-Hexane	Ethyl Acetate	Ethanol	Methanol
Flavonoid	HCl	-	+	+	+
Terpenoid	Liebermen Burchard	+	+	+	+
Steroid	Liebermen Burchard	-	+	+	-
Saponin	NaOH 25%	+	+	+	+
Tannin	$FeCl_3$	-	+	+	+
	Mayer	-	-	-	-
Alkaloid	DD	+	+	+	+
	Wagner	-	+	+	+

Flavonoids are phytochemical products known as polyphenols which are found in many herbs and plants, they are important anti-oxidative agents in counteracting free radicals. However, in the human digestive system, absorption of flavonoids in the intestine is very low and excretion is rapid and abundant through the process of urine excretion [12]. Terpenoids are the main components of essential oils which have been reported as anticancer, antimicrobial, anti-inflammatory, antioxidant and anti-allergic [13]. Tannin is a secondary metabolite of phenolic compounds which causes a bitter and astringent taste in L. acidissima fruit. It can react and coagulate proteins or other organic compounds containing amino acids and alkaloids.

Previous research results reported various potential benefits of L. acidissima for health due to its high phenolic content.

L. acidissima fruit extract was studied to have antioxidant properties by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST) and reducing lipid peroxidation in serum, liver and muscle tissue. Anti-inflammatory activity of methanol extract of L. acidissima by inhibiting carrageenan-induced paw edema and cotton pellet granuloma formation in animal models [14].

Results from GC-MS analysis on L. acidissima fruit extracts obtained using n-hexane, ethyl acetate, ethanol, and methanol as solvents, respectively, have been presented in Table 2. GC-MS analysis revealed the dominant presence of fatty acids in n-hexane and ethyl acetate extracts of L. acidissima fruit. The contents of oleic acid, n-hexadecanoic acid, palmitoleic acid, and octadecanoic acid were dominant in n-hexane and ethyl acetate extracts. Sterol groups such as γ-sitosterol, stigmasterol, ergost-5-en-3-ol, (3beta.) were identified in the ethyl acetate and ethanol extracts, but both had larger peak areas in the latter. As for the methanol extract, its content was rich in 2-pentanone, 4- hydroxy-4-methyl-, oleic acid and 9-octadecenoic acid (Z)-methyl ester.

Table 2. GC-MS analysis of *L.imonia acidissima* fruit extracts

Peak	RT (min)	Area	Area (%)	Height	Height (%)	A/H	SI	Name
***n*-Hexane**								
1	4.026	1290051	1.67	350024	1.39	3.69	93	2-Pentanone, 4-hydroxy-4-methyl-
2	7.975	484810	0.63	90085	0.36	5.38	81	1,3,5-Triazine-2,4,6-triamine
3	8.080	530913	0.69	224030	0.89	2.37	96	Nonanal
4	8.918	1820977	2.35	307716	1.22	5.92	95	4H-Pyran-4-one, 2,3-dihydro-3,5- dihydroxy-6- methyl-
5	9.055	555830	0.72	128872	0.51	4.31	63	Methyl 6-O-acetyl-2-deoxy-3,4-di- methyl-2(N-methylacetamido)-D-6
6	10.622	426708	0.55	149196	0.59	2.86	94	2-Decenal, (Z)-
7	12.440	199484	0.26	109242	0.43	1.83	96	3-Hexadecene, (Z)-
8	13.980	220811	0.29	100711	0.4	2.19	78	Cyclohexanol, 4-(1,1-dimethylethyl)-
9	14.792	904124	1.17	311120	1.23	2.91	97	Dodecanoic acid

10	15.133	753792	0.97	409127	1.62	1.84	95	1-Pentadecene
11	15.192	1182346	1.53	339223	1.34	3.49	97	Diethyl Phthalate
12	16.203	579182	0.75	204104	0.81	2.84	91	1,4-Dimethyl-7-(prop-1-en-2-yl)decahydroazulen-4-ol
13	17.212	2179207	2.81	909208	3.6	2.4	97	Tetradecanoic acid
14	17.549	1285829	5.82	674430	6.27	4.68	95	1-Heptadecene
15	18.046	250303	0.32	131106	0.52	1.91	95	Neophytadiene
16	18.339	422893	0.55	162596	0.64	2.6	97	Pentadecanoic acid
17	18.950	230272	0.3	102256	0.4	2.25	85	7,9-Di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione
18	19.234	992206	1.28	309988	1.23	3.2	96	Palmitoleic acid
19	19.459	15495338	21.7	4625686	20.78	7.78	96	n-Hexadecanoic acid
20	20.102	1512594	3.02	705633	3.92	5.03	80	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone
21	20.835	717573	0.93	329487	1.3	2.18	92	7-Octadecenoic acid, methyl ester
22	21.075	253744	0.33	137407	0.54	1.85	96	Methyl stearate
23	21.281	20183837	26.55	4510577	18.27	8.05	93	Oleic acid
24	21.472	4462029	5.76	1517854	6.01	2.94	92	Octadecanoic acid
25	21.733	1997515	2.58	933828	6.51	4.34	97	1-Hexacosene
26	22.751	479321	0.62	128722	0.51	3.72	89	Stearic acid, 2-hydroxy-1-methylpropyl ester
27	24.451	2319930	3	819083	3.24	2.83	60	2,3-Dihydro-7-methyl-5-phenyl-1H-1,4-benzodiazepine-2-thione
28	24.788	6007905	7.76	2644966	10.47	2.27	98	Bis(2-ethylhexyl) phthalate
29	25.238	1096397	1.42	489300	1.94	2.24	97	n-Tetracosanol-1
30	26.024	231286	0.3	104194	0.41	2.22	95	Phenethyl tetradecanoate
31	26.943	699965	0.9	289246	1.14	2.42	95	Eicosyl trifluoroacetate
32	29.051	377799	0.49	130450	0.52	2.9	96	Docosyl trifluoroacetate
Ethyl acetate								
1	4.026	1147409	1.22	361516	1.44	3.17	94	2-Pentanone,4-hydroxy-4-methyl-
2	9.318	555866	0.59	156549	0.62	3.55	97	endo-Borneol
3	14.299	225843	1.55	92450	1.57	9.83	96	Dodecanoic acid, methyl ester
4	15.152	312764	1	149042	1.96	3.93	97	1-Heptadecene
5	15.254	639286	0.68	134335	0.54	4.76	94	Diethyl Phthalate
6	16.763	780446	0.83	344350	1.37	2.27	96	Methyl tetradecanoate
7	17.238	2887656	3.07	903100	3.6	3.2	97	Tetradecanoic acid
8	18.368	425866	0.45	153743	0.61	2.77	97	Pentadecanoic acid
9	19.250	1053404	1.12	324946	1.29	3.24	96	Palmitoleic acid
10	19.480	18073289	23.24	5282675	28.65	7.39	96	n-Hexadecanoic acid
11	19.743	1188461	2.34	512879	3.62	4.88	93	1-Nonadecene
12	20.321	410022	0.44	141748	0.56	2.89	93	Hexadecanoic acid, 2-hydroxy-, methyl ester
13	20.843	4721344	5.95	2025976	9.84	4.33	91	9-Octadecenoic acid (Z)-, methyl ester
14	21.083	452217	0.48	229704	0.92	1.97	97	Methyl stearate
15	21.306	27056214	28.74	5517403	21.99	4.9	93	Oleic Acid
16	21.479	4561692	4.85	1201313	4.79	3.8	94	Octadecanoic acid
17	22.765	381678	0.41	89801	0.36	4.25	86	Stearic acid, 2-hydroxy-1-methylpropyl ester
18	23.561	619551	0.66	295344	1.18	2.1	97	1-Hexacosene
19	23.852	1531873	2.66	270963	2.07	9.53	81	Ergost-5-en-3-ol, (3.beta.)-
20	24.325	376120	0.4	91201	0.36	4.12	76	trans-13-Octadecenoic acid
21	24.572	3968961	4.22	581062	2.32	6.83	93	Stigmasterol
22	24.792	1102119	1.17	441685	1.76	2.5	97	Bis(2-ethylhexyl) phthalate
23	25.241	475463	0.51	212782	0.85	2.23	96	Octacosanol
24	26.013	7115676	7.56	917396	3.66	7.76	91	.gamma.-Sitosterol
25	26.358	1469050	1.56	216504	0.86	6.79	82	.beta.-Amyrone
26	26.947	346078	0.37	128214	0.51	2.7	92	1-Heptacosanol
27	27.506	2108640	2.24	347504	1.38	6.07	94	Lup-20(29)-en-3-one
28	28.286	528979	0.56	87387	0.35	6.05	86	Lupeol
29	29.376	873481	0.93	134625	0.54	6.49	91	.gamma-Sitostenone
Ethanol								
1	4.025	98941	0.56	46432	1.75	2.13	81	(3-Methyl-oxiran-2-yl)-methanol
2	4.090	1153202	6.51	165060	6.21	6.99	95	2-Pentanone,4-hydroxy-4-methyl-
5	19.454	572757	5.64	158349	7.26	5.7	96	n-Hexadecanoic acid
7	20.853	145684	0.82	56767	2.14	2.57	88	9-Octadecenoic acid(Z)-, methyl ester
8	21.264	775749	4.38	137042	5.16	5.66	95	Oleic Acid
9	21.481	306079	1.73	61543	2.32	4.97	91	Octadecanoic acid
10	23.871	2056710	11.61	205671	7.74	10	90	Ergost-5-en-3-ol(3.beta)
12	24.584	2365335	13.35	318043	11.96	7.44	92	Stigmasterol
13	24.803	1404841	7.93	346949	13.05	4.05	97	Bis(2-ethylhexyl) phthalate
16	26.029	4894046	27.62	577295	21.72	8.48	93	.gamma.-Sitosterol
17	26.995	767734	4.33	68537	2.58	11.2	80	Cholest-4-en-3-ol
21	28.307	207121	4.17	27528	4.04	7.52	80	Lupeol
22	29.389	613093	3.46	81789	3.08	7.5	91	.gamma.-Sitostenone
Methanol								
1	4.025	192944	3.03	44201	2.5	4.37	77	2-Pentanone,4-hydroxy
2	4.126	948524	14.9	140408	7.93	6.76	96	2-Pentanone,4-hydroxy-4-methyl-
3	4.288	709854	11.15	187178	10.58	3.79	98	Ethylbenzene
4	4.419	285593	4.49	69271	3.91	4.12	97	o-Xylene
5	19.045	363617	5.71	159297	9	2.28	96	Hexadecanoic acid, methyl ester
6	19.464	463263	7.28	132293	7.48	3.5	96	n-Hexadecanoic acid
7	20.794	198858	3.12	92765	5.24	2.14	94	9,12-Octadecadienoic acid

8	20.854	802629	12.61	307505	17.38	2.61	91	9-Octadecenoic acid (Z)-, methyl ester
9	21.091	81500	1.28	38558	2.18	2.11	94	Methyl stearate
10	21.272	912317	14.33	155875	8.81	5.85	95	Oleic Acid
11	21.492	190747	3	47496	2.68	4.02	85	Octadecanoic acid
12	24.805	929645	14.61	341534	19.3	2.72	97	Bis(2-ethylhexyl) phthalate

Previously, five main fatty acids have been identified in L. acidissima, namely palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid [9]. Monounsaturated fatty acids such as oleic acid are considered effective in lowering low-density lipoprotein (LDL) cholesterol levels thereby reducing the risk of coronary heart disease and preventing insulin resistance [9]. Oleic acid exhibits analgesic and anti-inflammatory activity by downregulating the expression of pro-inflammatory cytokines such as tumor necrotic factor alpha (TNF-α), interleukin 6 (IL-6) and nitric oxide (NO), as well as the production of free radicals in macrophage-stimulated lipopolysaccharide cells. Meanwhile, polyunsaturated fatty acids could reduce pro-inflammatory mediators or downregulate inflammatory cytokines such as IL-6, TNF-α, and monocyte chemotactic protein-1 (MCP-1) [15]. The compound n-hexadecanoic acid has been considered to have antioxidant and anti-inflammatory activities [16], [17] as well as an inhibiting activity against xanthine oxidase (XO) [18].

The results of previous in vitro studies have shown that triterpenoids exhibit potential anti-UA effects through their anti-inflammatory effects, while tannins exhibit a dual effects through UA formation inhibition and uricosuric action [19]. In this present study, the presence of triterpenoids and related compounds present illustrates the extract potentials as an anti-inflammatory and antioxidant which are important in treating hyperuricemia and gouty arthritis. Antioxidants prevent the oxidation of molecules in cells and protecting healthy cells from damage caused by free radicals and unstable and highly reactive molecules produced as by-products of uric acid metabolism.

3.2. Updated Literature Review

Sitosterol and its glycosides and stigmasterol are reported to have anti-inflammatory and immune-modulating activities. Although sitosterol failed to reduce serum UA levels in hyperuricemic rats, it was able to reduce leg swelling caused by monosodium urate crystals [20]. Based on the literature search, fatty acids along with other small phytocompounds are potential for treating gout, where the data have been summarized in Table 2.

Table 2. Summary of *L. acidissima* compounds with various extraction methods and their activities

Part of Use	Active Compound	Activity	Methods	Reff
Fruit pulp	Alkaloids, phenolics, flavonoids, steroids, tannins, saponins, triterpenoids, and glycosides	Antimicrobial	Methanolic and aqueous extract	[7]
Fruit pulp	Alkaloids	Anaesthetic and spasmolytic	Methanolic axtract	[21]
	Saponins	Reduce cholesterol level and enhance immunity-		
	Phenols	Antioxidant		
	Flavonoids	Anti-proliferative, enzyme inhibition, antibacterial		
Rip Fruit	Phenolic compounds	Antiproliferative	Methanol extract	[22]
Leaves	Zinc oxide nanoparticles	Antimicrobial	Aqueous extract	[23]
Pulp powder	Beta-carotene, thiamine and riboflavin, riboflavin, citric acid, oxalic acid , malic acid, tannin	Antioxidant and neuroprotective	Soxhlet-extracted with methanol	[24]
Fruit Pulp	Alkaloid, saponin, fenol plafanoid	Antispermatogenic and testicular antisteroidogenic activities	Ethanolic extract	[25]
Leaves	Coumarin (luvangetin, xanthotoxin, marmesin); triterpenoid (lipeol, limonin); sitosterol-O-B-D-glucoside),	Antioxidant	Methanolic extract (FL-7) and chloroform	[26]
	essential- oil (methyl chavicol, linalool)			
Bark	Marmesin	Hepatoprotective	Methanolic extract	[27]
Pericarp	Volatil flavours; free fatty acid	Antioxidant potential -	Methanolic extract	[28]
Fruit	Stigmasterol, citric acid, alkaloids, coumarins, fatty acids, scoparone, xanthotoxin	Anticancer activity on human breast cancer cell line, hepatoprotective, anti-spermatogenic	Ethanolic extract	[8]
		Anti-tumour, wound healing and anti- oxidant, anti diabetic	Metahnolic extract	

Previous studies have proven the activity of several plants belonging to the same family as L. acidissima (rutaceae). Fruit juice and lemon extract significantly decreased serum uric acid levels in human and rat subjects. The underlying mechanism suggests that lemon may lower serum uric acid independent of xanthine oxidase inhibition [29]. Apple juice intake has been shown to reduce plasma uric acid in fructose-induced model [30]. Oranges, grapefruit, and lemon juice were

reported to increase urinary citrate levels, acting to prevent kidney stone formation [31]. In another study, administration of orange and lemon juices prevent the formation of potassium oxalate and lowers uric acid [32].

4. CONCLUSION

The extract of L. acidissima fruit is predominated by fatty acid contents such as oleic acid, n-hexadecanoic acid, palmitoleic acid, and octadecanoic acid. Phytosterols were also observed in ethyl acetate and ethanol extracts from of L. acidissima fruit, namely -sitosterol, stigmasterol, ergost-5-en-3-ol, (3beta.). Based on literature review, bioactive compounds from L. acidissima plant are varied including alkaloids, phenolics, flavonoids, steroids, tannins, saponins, triterpenoids, and glycosides. Previous reports have suggested the potent bioactivities of L. acidissima plant including those related to antihyperuricemic and antiarthritic activities.

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